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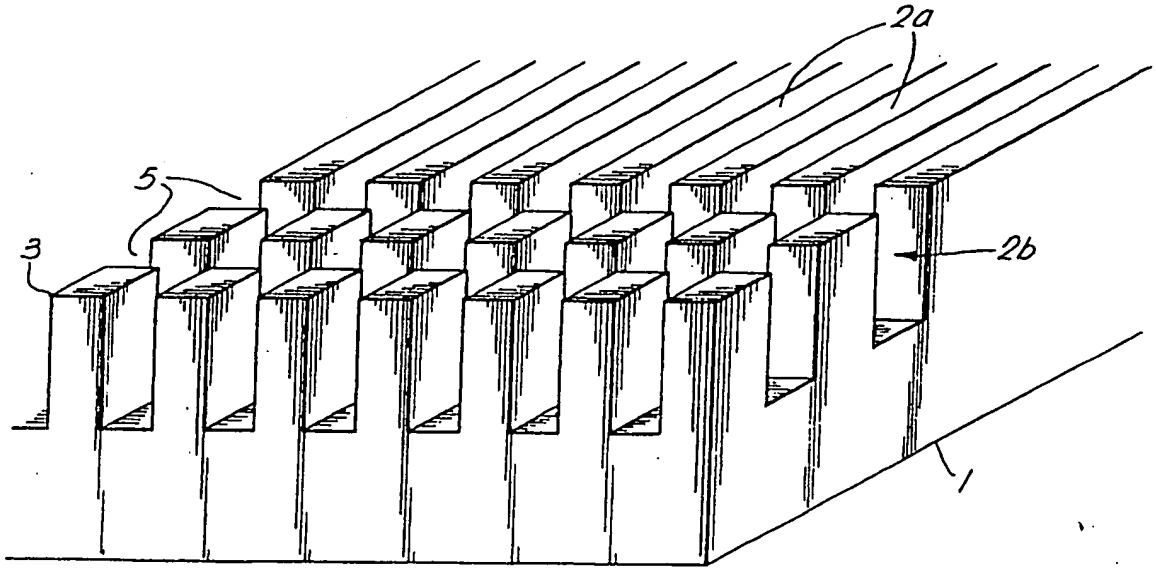

(57) Abstract

The present invention provides a device for the replication of arrays of cell colonies comprising a plate with a large number of small projections projecting therefrom. The projections being formed by removing, preferably by machining, the material between the projections, thus enabling a high density of projections to be achieved.

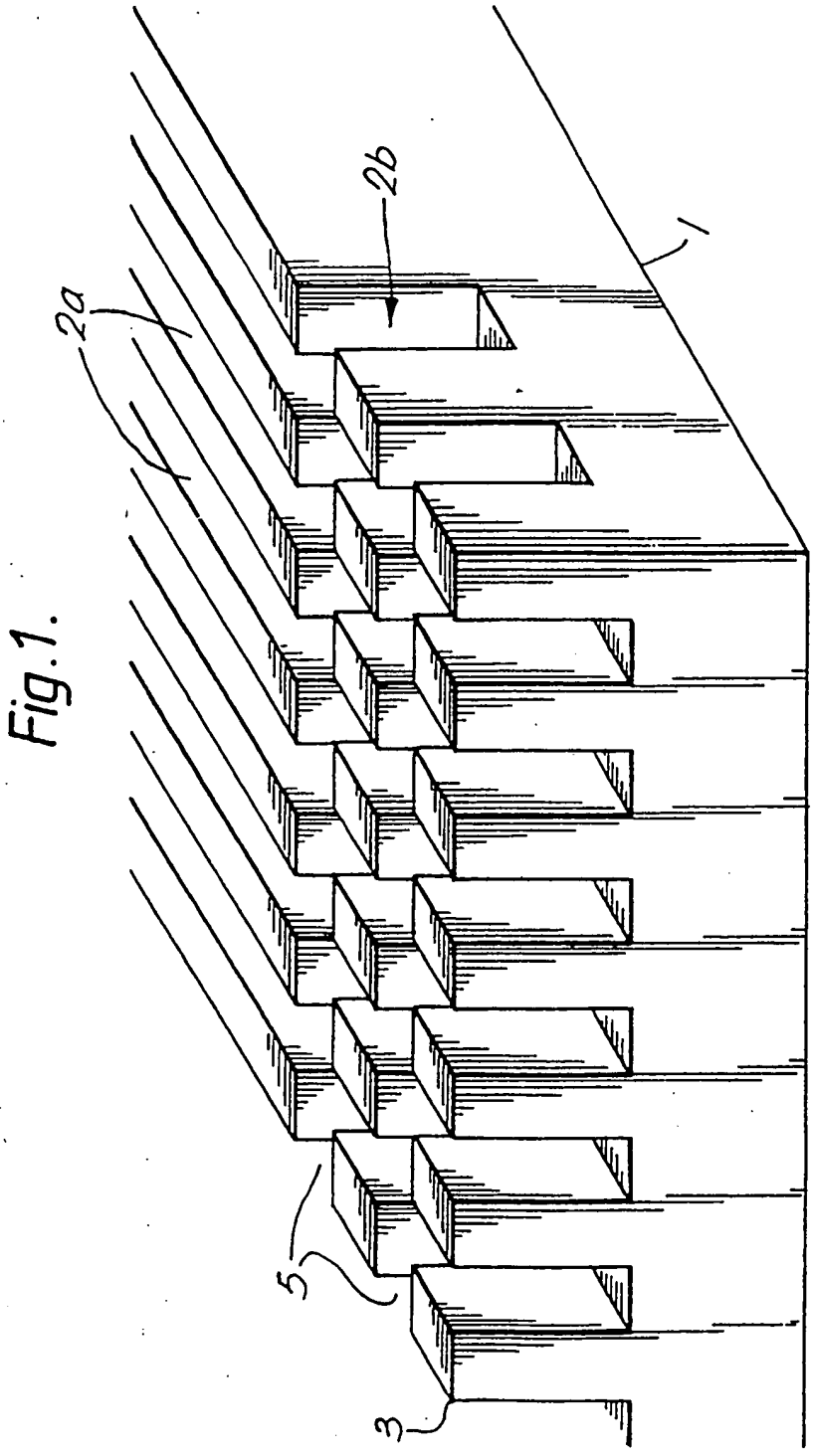

Fig.1.

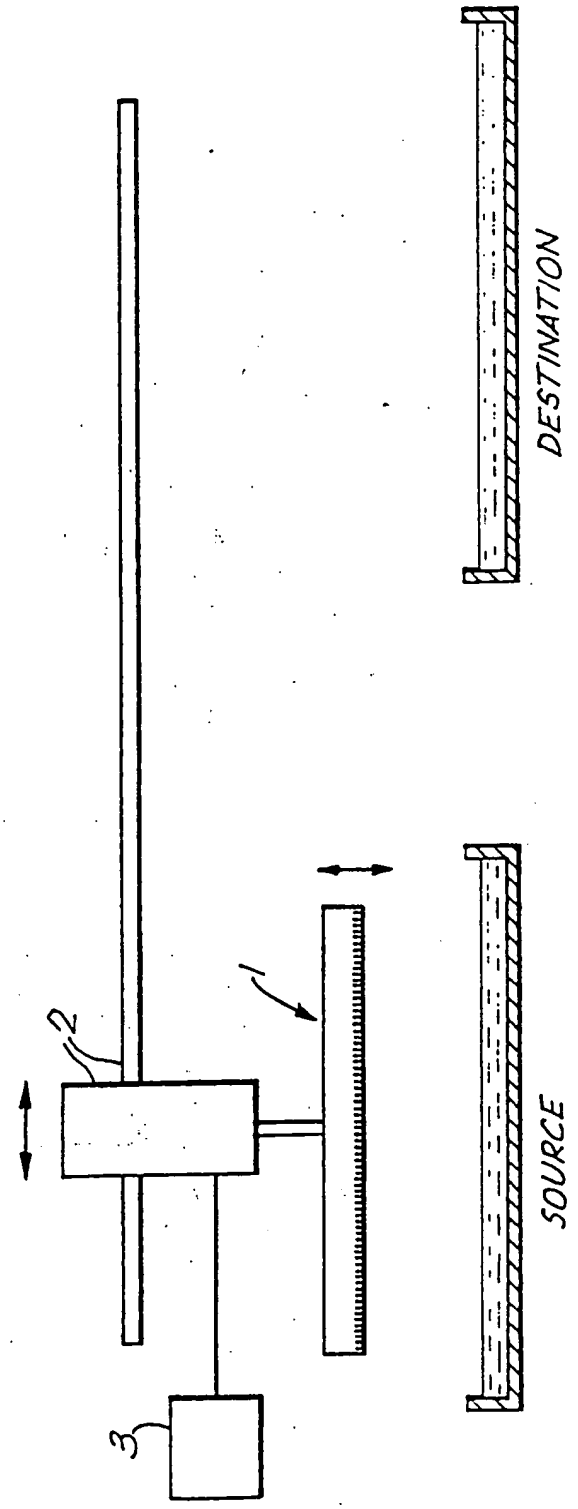

Fig.2.

SAMPLE MATERIAL TRANSFER DEVICE

The present invention relates to a device for removing material samples from a body of semi-rigid material such as agar, in particular for transferring cells from one culture plate to another, particularly when a large number of colonies having different characteristics are grown on the same plate.

In microbiology colonies of cells having various characteristics are nurtured in a layer of agar and nutrient on a culture plate. In order to conduct experiments on the cells it is necessary to transfer them from within the layer of agar to the surface. This has previously been done using tweezers, or some such similar probe, and which are dipped into the agar at the site of a cell colony which will hopefully cause a cell or cells to adhere to the tweezers. The tweezers are then gently touched to the surface of an agar layer on another culture plate. The intention is that a cell or cells will then be deposited on the surface of the second culture plate and grown into a new colony.

This process is laborious, inefficient and unreliable. These drawbacks are particularly manifest in molecular biology applications in which the colonies to be replicated are a large array of colonies in a culture plate; in an example, each colony has a different section of the human genome. In such applications it is vital that the colonies of the copy have an exactly corresponding position to that of their originals and that there is no contamination of one colony by its neighbours. If the transfer is performed manually the process will be very laborious and time consuming and prone to error.

According to the present invention there is provided a sampling device for removing material samples from a body of a semi-rigid medium such as agar comprising a sampling face provided with a multiplicity of sampling

projections having a high packing density per square centimetre of the sampling face, the projections being integrally formed with a substrate which maintains them in rigidly spaced relationship with interstices therebetween to admit medium from the body on insertion of the projections into the body of the medium.

The invention also provides a transfer apparatus for transferring colonies of microorganisms from between semi-rigid culture comprising respective holders of source- and destination medium and a transfer device for sampling colonies in the source medium and transferring the sample to the destination medium, the device comprising a sampling face provided with a multiplicity of sampling projections having a density per unit area of the sampling face substantially higher than the density of the colonies per unit area of the source-medium, the projections being integrally formed with a substrate which maintains them in rigidly spaced relationship with interstices therebetween to admit medium from the body on insertion of the projections into the body of the medium.

The invention further provides a method of replicating an array of cell colonies using a device or apparatus according to the invention, said method comprising the steps of:

placing said device on a culture plate containing the array to be replicated so that a cell or cells from each colony adheres to a projection or projections of said device, and touching said device to the surface of a fresh culture plate so that at least some of said cell or cells are transferred to the surface of said fresh culture plate.

Conveniently, the projections are square in cross-section. The projections and substrate may be machined from a block of metal or moulded from plastics material.

As will become apparent from the following description, the invention provides an effective means of

transferring cell colonies from one medium to another.

The present invention will be further described hereinafter with reference to the following description and the accompanying drawings in which:

Figure 1 is a perspective view of a corner of a partially completed device according to the present invention; and

Figure 2 shows schematically an automatic apparatus for the transfer of cell colonies including a completed device of Figure 1 (note that in Figure 2 the sizes of the projections are exaggerated for clarity).

Figure 1 shows a device 1 according to the invention in the form of a block in which two perpendicular sets 2a, 2b of evenly spaced parallel grooves are cut, creating sets of parallel walls. The sets of grooves define a multiplicity of rigid pillars or projections 3 arranged in a rectangular array, with the tips of the projections conforming to a flat plane.

The grooves may typically be of the order of 1-2 mm deep, and the projections 1-2 mm high. The grooves are a fraction of a millimetre width.

Suitably, several tens of the grooves are cut per centimetre creating projections a fraction of a millimetre square with several up to a thousand projections per square centimetre. The size and density of the projections is dictated in practice by the fineness to which the grooves can be cut by the fabrication technique used; in the case of a device made from metal, eg, aluminium, the grooves can be formed by milling. An impression may be taken of the machined block and used as a mould to produce relatively cheap, possibly plastic, devices. These might be made sufficiently cheaply so that they can be disposed after use avoiding the necessity to clean and sterilise the devices between uses and is requried if the device is to be re-used.

In one suitable construction, the pins are 1.2 mm apart (ie, the centre of the pins and the centre of the

milled grooves are at a 1.2 mm pitch). Using a typical 22 cm square transfer device size, this gives a 200 by 200 array of pins, ie, 40,000 pins per device.

Lower and upper limits on the pin spacing might be, respectively, 0.5 and 1.5 mm for typical applications. Typically, colonies are of the order of 1 mm across, but they are sometimes smaller than this. To get a pick-up rate of approximately 80% on small colonies, a pin spacing of 0.5 mm might be appropriate; in any event, the optimum spacing can be determined by simple experiment.

Typically there would be of the order of 10,000 colonies on a plate, each of the order of 1 mm across and comprising a cluster of single cells. It has been found by experiment that, using a sampling device with an array of 200 by 200 projections usually results in two or three projections each picking up cells from each colony where the projections are pressed into the agar medium and then withdrawn. Given that there are relatively large spaces between colonies, the high packing density of the projections means that during the sampling step there are always a few projections (say three or four) which are in the vicinity of each colony and therefore potentially capable of picking up cells from it. The high packing density of the projections also ensure that cells from different colonies are kept separate from one another.

The rigidity and packing density of the projections 3 are significant in that for accurate transfer of colonies it is desirable that the projections 3 maintain their normal spaced relation throughout the operation, so that there is no smearing of the samples (as might occur if the projections deflected).

In the illustrated embodiments the grooves have a rectangular cross section, having been cut with a square tool, and hence the projections are flat-topped. However by using different shaped tools to cut the grooves pointed or

other shaped projections can be produced.

Other methods of forming the grooves, such as chemical etching, may also be used.

The use of the device is as follows. Arrays of cell colonies are grown in a layer of agar and nutrient on a culture plate. Each colony has cells of particular characteristics, each colony may contain a different random section of the human genome for example, and the colonies are identified by their position on the plate.

In order that the tests or experiments may be carried out on the cell colonies it is necessary that they are on the surface of an agar layer rather than embedded within it. It is also often advantageous to have multiple copies of the array of colonies for multiple tests and control purposes. In order to make such copies the device according to the present invention is employed.

Thus in use, the device is gently pressed with an even pressure against the source-medium (the one from which it is desired to transfer the cell colonies); the projections retain material from the source-medium on withdrawal of the device from the source medium. The device is then moved to the destination medium, eg, a fresh agar plate, and the colonies 'planted' by pressing the projections onto it. The source medium may then be allowed to regenerate for a short period after which further copies may be taken. The cells deposited on the surface of the destination culture plate multiply to form new colonies.

In some applications, it is not necessary to wait for this regeneration to occur, since each projection removes multiple cells per colony, and multiple sampling/transfer/deposition steps may be carried out in succession to make multiple identical replicas of the colonies onto either different destination plates or different areas of a large plate.

The device according to the present invention

allows quick and reliable replication of a colony array. The high density of projections ensures that the copies have the same distribution as the original colonies allowing them to be readily identified.

The device 1 may be fitted to a handle or similar for manual use. Alternatively, it may be provided with an attachment for fitting it to an automated transport device 2 (for example an XYZ axis controller) which can be programmed to carry out a sampling motion relative to the source medium to pick up the samples with the device, a transfer motion to move the device to the destination medium and a depositing motion relative to the destination medium to deposit the samples, under the control of a controller 3.

The pick-up face of the device need not be rectangular or square as shown but instead could be circular, for example, if it is desired to pick-up colonies from circular agar plates.

The pick-up face of the device need not be flat. In one alternative, it could be gently convex (eg, conforming to part of the surface of a cylinder) so that a gently rolling action could be used for the pick-up operation. This could be advantageous because sometimes, with a flat pick-up face, there is imperfect picking-up of the colonies from certain areas of the agar and this may be ameliorated using a convex pick-up face. Equally, the pick-up device could be cylindrical, with the pins on its peripheral surface, and mounted for rotation in a holder, preferably biased by a gently-acting return spring to a reference orientation in the holder. This could be used to pick up colonies from a long thin area of an agar plate by rolling the device over the surface of the agar; after the rolling operation, the spring would then return the pick-up device to its reference orientation in the holder, enabling the depositing operation to be carried out (by a similar rolling action) in a manner which sets the colony samples

down in the same layout as the original samples.

CLAIMS

1. A sampling device for removing material samples from a body of a semi-rigid medium such as agar comprising a sampling face provided with a multiplicity of sampling projections having a high packing density per square centimetre of the sampling face, the projections being formed integrally with a substrate which maintains them in rigidly spaced relationship with interstices therebetween to admit medium from the body on insertion of the projections into the body of the medium.

2. A device according to claim 1 wherein the projections are defined by a closely packed array of perpendicular sets of grooves on the sampling face.

3. A device according to claim 1 or 2 wherein the free ends of the projections conform to a flat plane.

4. A device according to claim 1, 2 or 3 wherein the projections and substrate are machined from a block of metal.

5. A device according to claim 1, 2 or 3 wherein the projections and substrate are moulded from a plastics material.

6. A device according to any one of claims 1 to 5 wherein the projections are square in cross-section.

7. A transfer apparatus for transferring colonies of microorganisms from between semi-rigid culture comprising respective holders of source- and destination medium and a transfer device for sampling colonies in the source medium and transferring the sample to the destination medium, the

device comprising a sampling face provided with a multiplicity of sampling projections having a density per unit area of the sampling face substantially higher than the density of the colonies per unit area of the source-medium, the projections being integrally formed with a substrate which maintains them in rigidly spaced relationship with interstices therebetween to admit medium from the body on insertion of the projections into the body of the medium.

8. An apparatus according to claim 7, wherein the projections are defined by a closely packed array of perpendicular sets of grooves on the sampling face.

9. An apparatus according to claim 7 or 8 wherein the free ends of the projections conform to a flat plane.

10. An apparatus according to claim 7, 8 or 9 wherein the projections and substrate are machined from a block of metal.

11. An apparatus according to claim 7, 8 or 9 wherein the projections and substrate are moulded from a plastics material.

12. An apparatus according to any one of claims 7 to 11 wherein the projections are square in cross-section.

13. An apparatus according to any one of claims 7 to 17 and including an automated transfer mechanism operative to drive the sampling device through a sampling motion relative to the source-medium, a transfer motion between the holders of the source- and destination-medium and a sample depositing motion relative to the destination medium.

14. A transfer device constructed and arranged to

operate substantially as hereinbefore described with reference to and as illustrated in the accompanying drawings.

15. A method of replicating an array of cell colonies using a device according to any one of claims 1 to 6 and 14 or the apparatus of any one of claims 7 to 13, said method comprising the steps of:

placing said device on a culture plate containing the array to be replicated so that a cell or cells from each colony adheres to a projection or projections of said device, and touching said device to the surface of a fresh culture plate so that at least some of said cell or cells are transferred to the surface of said fresh culture plate.

16. A method of replicating an array of cell colonies substantially as hereinbefore described with reference to and as illustrated in the accompanying drawings.